Listing of the claims

1. (previously presented) A method for selectively labeling or tagging one or more phosphate groups in a natural or synthetic peptide or protein groups which also contains one or more carboxylic acid groups or esters thereof which method comprises the steps of:

   a. reacting the natural or synthetic peptide or protein with a protective group that is an amine that reacts to protect the one or more phosphate groups therein by forming phosphoramide bonds and to protect any carboxylic acid groups and esters thereof therein by forming amide bonds;

   b. treating the protected peptide or protein under conditions which selectively substantially cleave the phosphoramide bonds, without substantially cleaving the amide bonds to regenerate one or more free phosphate groups in the peptide or protein; and

   c. reacting the one or more free phosphate groups in the peptide or protein, in which the carboxylic acids groups remain protected, with a label or tag comprising a functional group that reacts with a phosphate group to thereby generate a peptide or protein in which the phosphate groups are selectively labeled or tagged.

2. Canceled

3. (previously presented) The method of claim 1 wherein the label or tag is a solid phase material and the one or more free phosphate groups of the peptide or protein are covalently linked to the solid phase material directly or indirectly through a linker moiety.

4. (previously presented) The method of claim 1 wherein the amino group of the protective group that is an amine is reacted with the one or more phosphate groups of the peptide or protein using a carbodiimide catalyzed reaction.

5. (previously presented) The method of claim 3 wherein the one or more free phosphate groups of the peptide or protein are reacted with a linker group wherein the linker group comprises a phosphate reactive group and a second reactive group that functions for forming a covalent bond to the solid phase material.

6. (original) The method of claim 5 wherein the second reactive group is a sulfhydryl reactive group.

7. (currently amended) The method of claim 1 wherein the ~~protecting~~ protective group is ethanolamine.

8. (previously presented) The method of claim 7 wherein in step b the protected peptide or protein is treated with trifluoroacetic acid to selectively regenerate one or more free phosphate groups.

9. (previously presented) The method of claim 1 wherein the one or more free phosphate groups are reacted with a linker which contains a sulfhydryl group or which contains a latent reactive group that can be transformed into a sulfhydryl group.

10. (previously presented) The method of claim 9 wherein the one or more free phosphate groups are first reacted with cystamine and thereafter one or more free sulfhydryl groups are generated by reduction of the disulfide group of the cystamine.

11. (currently amended) The method of claim 10 wherein the ~~reducing agent is DTT~~ disulfide group of the cystamine is reduced using DTT.

12. (previously presented) The method of claim 9 wherein in step c the peptide or protein is covalently attached to a solid support material through reaction with the sulfhydryl group of the linker.

13. (original) The method of claim 12 wherein the solid support material is glass beads with immobilized iodoacetyl groups.

14. (previously presented) The method of claim 1 wherein the natural or synthetic peptide or protein is obtained from a tryptic digest.

15. (original) The method of claim 1 wherein the label or tag is a radiolabel, a fluorescent label, a colorimetric label or an affinity label.

16. (previously presented) The method of claim 1 wherein the label or tag is an affinity label.

17. (original) The method of claim 1 wherein the label is a reactive label.

18. (previously presented) The method of claim 1 further comprising a step of detecting the selectively labeled or tagged peptide or protein.

19. (previously presented) The method of claim 18 wherein the selectively labeled or tagged peptide or protein is detected by detection of the label or tag.

20. (previously presented) The method of claim 18 wherein the label or tag is an affinity label and the selectively labeled or tagged peptide or protein is detected by binding to a capture reagent.

21. (previously presented) The method of claim 1 wherein step c comprises attaching the peptide or protein having one or more free phosphate groups to a solid support or binding the peptide or protein having one or more free phosphate groups to a capture reagent.

22. Canceled

23. (currently amended) ~~The~~A method ~~of claim 22 wherein, in step a, the carboxylic acid groups or esters thereof of the peptides or proteins are protected~~ for detecting one or more phosphopeptides or phosphoproteins in one or more samples containing a mixture of peptides, proteins or both, which comprises the steps of:

a. selectively protecting carboxylic acid groups of the peptides or proteins in the one or more samples by initial reaction with a protecting group that protects both carboxylic acid groups, esters thereof and phosphate groups in the peptides and proteins followed by selective deprotection of the phosphate groups in the peptides and proteins such that any phosphate groups in the peptides or proteins are unprotected;

b. selectively labeling the unprotected phosphate groups in the peptides or proteins in the sample with a label having a functional group that reacts directly or indirectly with a phosphate group; and

c. detecting the peptides or proteins carrying the selective label of step b to detect the one or more phosphopeptides or phosphoproteins in the sample.

24. (previously presented) The method of claim 23 wherein the carboxylic acid groups, esters thereof and phosphate groups of the peptides or proteins are protected with an amine group that forms amide bonds with carboxylic acid

groups and esters thereof and phosphoramide bonds with phosphate groups and wherein the protected phosphate groups are thereafter selectively deprotected by cleavage of the phosphoramide bonds.

25. (previously presented) The method of claim 24 wherein the amide bonds and phosphoramide bonds are formed by carbodiimide-catalyzed condensation reactions.

26. (previously presented) The method of claim 25 wherein the phosphate groups are deprotected without cleavage of the amide bonds by treatment with mild acid.

27. (currently amended) The method of claim ~~22~~ 23 wherein the label is a radiolabel, a fluorescent label, a colorimetric label or an affinity label.

28. (currently amended) The method of claim ~~22~~ 23 wherein the label is an affinity label and the selectively labeled phosphopeptides or phosphoproteins are detected by binding to a corresponding capture reagent.

29. (currently amended) The method of claim ~~22~~ 23 wherein the label is a reactive label.

30. (original) The method of claim 29 wherein the reactive label carries a reactive group that can form a covalent bond to a solid phase material.

31. (original) The method of claim 29 wherein the reactive label carries a latent reactive group.

32. (currently amended) The method of claim ~~22~~ 23 further comprising the step of separating selectively labeled phosphopeptides or phosphoproteins from the mixture of peptides or proteins in a sample prior to detection step c.

33. (previously presented) The method of claim 32 wherein the label carries a reactive group that can form a covalent bond to a solid phase material and wherein the selectively labeled phosphopeptides or phosphoproteins are separated by first covalently attaching the labeled phosphopeptides or phosphoproteins to the solid phase material, then washing the solid support to remove peptides or proteins that are not covalently attached to the support and thereafter releasing covalently attached phosphopeptides or phosphoproteins from the solid phase material.

34. (previously presented) The method of claim 33 wherein the phosphopeptides or phosphoproteins released from the solid phase material are detected using mass spectrometric techniques.

35. (currently amended) The method of claim 34 wherein tandem mass spectrometry is used to detect the phosphopeptides or phosphoproteins released from the solid phase.

36. (currently amended) The method of claim 35 wherein tandem mass spectrometry is further used to determine the amino acid sequence of the phosphopeptides or phosphoproteins released from the solid phase and the precise position of the phosphorylated amino acid within the sequence of the phosphopeptides or phosphoproteins.

37. (currently amended) The method of claim ~~22~~ 23 for detecting one or more phosphopeptides or phosphoproteins in two or more samples wherein differentially isotopically labeled protecting groups for carboxylic acids or esters thereof are employed with different samples.

38. (previously presented) The method of claim 37 for detecting one or more phosphopeptides or phosphoproteins in two or more samples wherein the labels employed in different samples are differentially isotopically labeled.

39. (previously presented) The method of claim 37 wherein tandem mass spectrometry is used to detect the one or more phosphopeptides or phosphoproteins and the relative amounts of phosphopeptides or phosphoproteins in the two or more samples are determined by measuring the relative amounts of the differentially isotopically labeled labels present.

40. (currently amended) The method of claim 39 wherein combined microcapillary liquid chromatography and tandem mass spectrometry are employed to detect the one or more phosphopeptides or phosphoproteins.

41. (previously presented) The method of claim 1 wherein the amine is a hydroxy amine.

42. Canceled

43. (currently amended) The method of claim 1 wherein the ~~protecting~~ protective group is a differentially isotopically labeled protecting group.

44. (currently amended) The method of claim 43 wherein the ~~protecting~~ protective group is a hydroxy amine.

45. (currently amended) The method of claim 43 wherein the ~~protecting~~ protective group is differentially isotopically labeled using deuterium.

46. (currently amended) The method of claim ~~22~~ 23 for detecting one or more phosphopeptides in two or more samples wherein differentially isotopically

labeled ethanolamine is used to protect the carboxylic acid groups of peptides in different samples.

47. (currently amended) The method of claim ~~22~~ 23 further comprising a step of determining the sequence of one or more phosphopeptides detected.

48. (original) The method of claim 47 wherein the sequence of the phosphopeptide is determined by tandem mass spectrometry.

49. (previously presented) The method of claim 48 wherein the samples are protein digests containing peptides and the sequence of the phosphopeptide detected is used to identify the protein from which the phosphopeptide is derived.

50. (currently amended) The method of claim ~~22~~ 23 in which the amount of one or more phosphoproteins in a sample is also determined by mass spectrometry, and which further comprises the step of introducing into a sample a known amount of one or more internal standards for each of the phosphoproteins to be quantitated.

51. (original) The method of claim 50 in which different samples represent proteins expressed in response to different environmental or nutritional conditions, different chemical or physical stimuli or at different times.

52. (currently amended) The method of claim ~~22~~ 23 wherein phosphopeptides or phosphoproteins in different samples are labeled with different fluorescent labels and the relative amounts of a labeled phosphopeptide or phosphoprotein in different samples can be measured by measuring the relative intensity of the fluorescence emission of a labeled phosphopeptide or phosphoprotein in different samples.

Claims 53-67 (canceled)

68. (previously presented) The method of claim 1 further comprising a step of protecting any amine groups in the peptide or protein prior to step a.

69. (previously presented) The method of claim 68 wherein the amine groups are protected with t-boc or f-moc.

70. (currently amended) The method of claim ~~22~~ 23 further comprising a step of protecting any amine groups in ~~the~~ peptides or proteins of the one or more samples prior to step a.

71. (currently amended) The method of claim 70 wherein the amine groups of ~~the~~ peptides or proteins of the one or more samples are protected with t-boc or f-moc.

Claims 72-73 (canceled)

74. (previously presented) The method of claim 21 wherein the peptide or protein having one or more free phosphate groups is present in a mixture containing peptides or proteins that do not contain phosphate groups and which further comprising a step of separating peptides or proteins in which the phosphate groups are selectively labeled or tagged from peptides or proteins that do not contain phosphate groups.

75. (previously presented) The method of claim 74 wherein the peptide or protein in which the phosphate groups are selectively labeled or tagged is attached to a

solid support and the peptide or protein attached to the solid support is separated from peptides and proteins not attached to the support.

76. (previously presented) The method of claim 74 wherein the peptide or protein in which the phosphate groups are selectively labeled or tagged comprises an affinity label which is attached to a capture reagent and the peptide or protein attached to the capture reagent is separated from peptides and proteins not attached to the capture reagent.

77. (currently amended) The method of claim ~~22~~ 23 for detection of one or more phosphopeptides.

78. (currently amended) The method of claim ~~22~~ 23 wherein the mixture of peptides and proteins of the one or more samples is obtained from a tryptic digest.

79. (previously presented) The method of claim 28 wherein the label is a reactive label.

80. (previously presented) A method for detecting one or more phosphopeptides or phosphoproteins in one or more samples containing a mixture of peptides or proteins which comprises the steps of:

a. reacting the peptides or proteins in the mixture with a protective group that is an amine that reacts to protect any phosphate groups therein by forming phosphoramide bonds and to protect any carboxylic acid groups and esters thereof therein by forming amide bonds;

b. treating the protected peptides or proteins in the mixture under conditions which selectively substantially cleave the phosphoramide bonds, without

substantially cleaving the amide bonds, to regenerate free phosphate groups in the peptide or protein;

c. selectively labeling the free phosphate groups in the peptides or proteins in the sample with a label having a functional group that reacts directly or indirectly with a phosphate group; and

d. detecting the peptides or proteins carrying the selective label of step c to detect the one or more phosphopeptides or phosphoproteins in the sample.

81. (previously presented) The method of claim 80 wherein the protective group is ethanol amine.

Amendments to the Drawings

Form PTO 948 indicated that the margins for Fig. 1 needed to be corrected. Accompanying this submission is a copy of Fig. 1 with corrected margins. The figure has been printed in landscape format. Applicant is submitting formal drawings for this application under separate cover.